Identification, Evaluation and Molecular Characterization of *Stylosanthes seabrana*--a Potential and Nutritious Range Legume Having Wider Applicability in India

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Introduction The *Stylosanthes* (Fabaceae) genus has wide distribution in tropical , subtropical and temperate regions of the Americas , Africa and South East Asia . In India , S*tylosanthes* is extensively utilized in pastoral , agropastoral and silvipastoral systems (Chandra et al . , 2006) . The characterization of new species in genus *Stylosanthes* is always demanding . The newly identified *S . seabrana* is one such species established well in India without specific rhizobium and is superior in nutritional quality namely essential amino acids . Importance of this species has been also emphasized in other countries like China , Australia and Colombia (Phailkaew et al . , 2004) . It is also better for leaf meal production and a suitable replacement of concentrate for livestock . However , limited numbers of lines in this species pose problems in its wider applications . Here , we report the recovery of ten new lines of *S . seabrana* from existing stocks of *S . scabra* utilizing STS markers which were further supported by morphological attributes . These lines were molecularly characterized using RAPD and STS markers to estimate the level of variability and genetic distance from *S . scabra* of which *S . seabrana* is one of the diploid progenitors (Liu and Musial , 1997) . These lines were also assessed for drought tolerance parameters like specific leaf area , osmolality , proline and soluble protein contents as better drought tolerance lines can be used in artificial synthesis of allo-tetraploid *S . scabra* (Tewari and Chandra , 2008) .

Materials and methods In total 48 accessions procured from ILRI , Ethiopia as *S . scabra* genetic stocks and nine known *S . seabrana* lines from Australia were established at Indian Grassland and Fodder Research Institute , Jhansi and were morphologically evaluated . Specific leaf area (SLA) was measured by randomly harvesting six trifoliate leaves . Osmolality was measured using a vapour pressure osmometer . Proline was estimated colorimetrically . Genomic DNA was isolated using buffer S (Liu and Musial , 1997) . RAPD and STS input binary data matrix was developed by entering 1 to presence or 0 to absence of bands . Dice similarity coefficient was processed for SAHN cluster analysis using UPGMA and tree display was followed for generation of dendrogram (NTSYS tree phenogram) .

Results and discussion Of the 48 accessions of *S . scabra* , 10 distinct lines were identified on the basis of early flowering , leaf shape and less hardiness of stem in comparison to *S . scabra* . However , in all these ten lines as in *S . scabra* an axis rudiment , a small appendage at the base of the pod or loment was observed . These characteristics clearly divided these lines from the existing stocks of *S . scabra* . When they were assessed with genome specific marker like sequence - tagged-sites (STS) , all displayed STS phenotypes of typical diploid species . With primer pairs SHST3F3/R3 all putative *S . seabrana* yielded single band of ~550 bp and *S . viscosa* of ~870 bp whereas both of these bands were observed in allo-tetraploid *S . scabra* . Since this primer pairs is known to amplify single or no band with diploid and two bands with tetraploid species , the amplification patterns corroborated that all newly identified *S . seabrana* lines were diploid in nature . Flow-cytometric analysis also indicated the proportional sharing of DNA content between *S . scabra* , and *S . viscosa* and recovered *S . seabrana* lines further indicated the identified lines as *S . seabrana* . Thirty-two RAPD and 17 STS primers indicated more than 92 % similarity between any two accessions of newly identified lines . Low levels of variability in osmolality , SLA , proline and protein content were also observed among the identified *S . seabrana* lines . Results indicated the utility of gene specific markers in identification of unexplored species of related forage crops .

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